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A Comparative Study of the Effect of Gallic Acid on Cancer Cells and Normal Cells.

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ABSTRACT

Several plants contain polyphenols that show beneficial effects for human health. The present report deals with the chemo-protective activity of gallic acid that is present abundantly in green tea. The activity of gallic acid has been studied on cancer cells and normal cells; many studies have reported the anti-oxidant properties of gallic acid. However, there are few reports of its anti-cancer activity on cell lines. The present study investigated the effect of gallic acid on cell proliferation and extent of DNA damage in colon cancer cell line and normal human lymphocytes. The study indicates that HCT15 cells are susceptible to gallic acid whilst the lymphocytes are not. The DNA damage due to gallic acid is found to be extensive in case of HCT15 cells but not significant in the case of lymphocytes. The outcome of the present study indicates that gallic acid is a promising anti-cancer agent with minimal toxicity in normal cells.
Keywords: Gallic acid, HCT15, lymphocytes, Comet assay.

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INTRODUCTION

Carcinogenesis is a multi-step process involving an imbalance of numerous factors which is one of the important areas of research. In spite of all the advances in cancer therapy, the search for the chemopreventive agents is still on as there has been a statistical increase in deaths due cancer related issues [1]. GLOBOCAN had reported an estimated 8.2 million cancer related deaths in 2012, which was 7.6 in 2008 [2].

Many epidemiological studies have confirmed the important role played by dietary compounds in reducing the risks of cancer. In fact, several studies have indicated that the populations in South East Asia have lower risk of cancer than their counter parts in the western countries. It is apparent that the food habits of these populations play a key role in preventing the disease [3]. More importantly, the use of conventional chemotherapeutic drugs is limited due to their life-threatening side-effects because of which polyphenols and other phytochemicals are being studied for possible use as chemotherapeutic agents [4, 5].

Polyphenolic compounds are a group of compounds found abundantly in plants. These are well known for possessing pharmacological properties such as anti-oxidant and anti-cancer activity [6]. Gallic acid is one among the most prominent polyphenols. In recent years, researchers have studied the activities of gallic acid as strong antioxidant, anti-mutagenic, anti-inflammatory and anticancer agents. The anticancer activity of gallic acid has been proved in different cancer cell lines like lung, prostate, cervical and colon [7]. Moreover, scientists were persuaded to proceed with studies of different derivatives of gallic acid. The indanone derivatives exhibited potential anticancer property against hormone-dependent breast cancer, liver and oral cancer cell lines [8]. In addition, the protective activities of antioxidants against the toxic chemicals are being studied *in vitro* with the help of cell culture [9]; and lymphocytes are considered ideal for studying toxicity and cytoprotectivity on a routine basis [10]. The present study has been initiated with the objective of evaluating the activity of the gallic acid on the cancer cell lines and normal cells. The result will further lead to the development of gallic acid as an anticancer drug and will be able to disclose its protective effect.

MATERIALS AND METHODS

Materials and Medium

All chemicals used were procured from Sigma Aldrich, USA. Reagents and medium involved in cell culture was from HiMedia, India. Growth medium was prepared by supplementing RPMI 1640 with 10% fetal bovine serum, penicillin (100 IU/ml) and streptomycin (100µg/ml) was used for cell culture.

Cell Culture

The human colorectal cancer cell line, HCT15, obtained from NCCS, Pune, was used. It was maintained in growth medium at 37^0C in a humidified 5% CO_2 incubator.

Lymphocyte Isolation

Lymphocytes were isolated from fresh blood using Histopaque 1077, Sigma Aldrich, USA. Fresh blood was drawn from a healthy person at the age of 25-30, who was not exposed to any drugs for 6 months. The viable lymphocytes were counted using trypan blue exclusion method in a Neubauer type Hemocytometer and the concentration of the cells was made up to 2×10^5cells/ml.

Treatment

The HCT15 cancer cells and lymphocytes were exposed to gallic acid at the concentration range from 5 µg/ml to 100 µg/ml. After the desired incubation time, 24 hr, the cells were collected for further studies. The untreated cells served as control.

Morphological Changes

After the treatment, cells were observed under inverted microscope and photomicrographs were taken for the analysis of any morphological changes in the cells.

MTT Assay

The MTT assay was performed using minor modification of Mosmann [11]. The treated cells were washed with PBS (phosphate buffer saline) and treated with MTT **(**3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide**).** After 5 hrs of incubation, DMSO (dimethyl sulfoxide) was used to dissolve the fromazan crystals formed resulting in purple color. This was read at 540 nm.

Comet assay

The Comet assay was executed according to Tice *et. al.* [12]. 10 µl of the treated and untreated cells resuspended in 1% low melting agarose in PBS was layered on the slide pre-coated with 1% normal melting agarose. The slides were incubated at 4^0C for 30 min. A third layer was applied with 1% normal melting agarose and again incubated at 4^0C for 30 min. Subsequently, the slides were kept in cold lysis buffer (10 mM Tris, 2.5 M NaCl, 100 mM EDTA, 1% N-lauroylsarcosine at pH 10, 1% Triton X-100 and 10% DMSO) [13] overnight at 4^0C. For unwinding of DNA these were then incubated in alkaline electrophoresis buffer (300 mM NaOH, 1 mM EDTA at pH 13) for 20 min. Later on, the slides were washed thrice with neutralizing buffer (0.4 M Tris HCl at pH 7.5) after electrophoresis at 0.8V/cm and 300mA for 20 min. 25 µM propidium iodide was used as stain after the slide were fixed with 75% ethanol and observed under fluorescent microscope. OPEN COMET was used to score 50 comets.

RESULTS

Morphological Changes:

The morphology of the cancer cells, observed under inverted phase contrast microscope, showed signs of apoptosis such as cell shrinkage, nuclear condensation and detachment from substratum, while no significant changes was observed in treated lymphocytes (Fig 1).

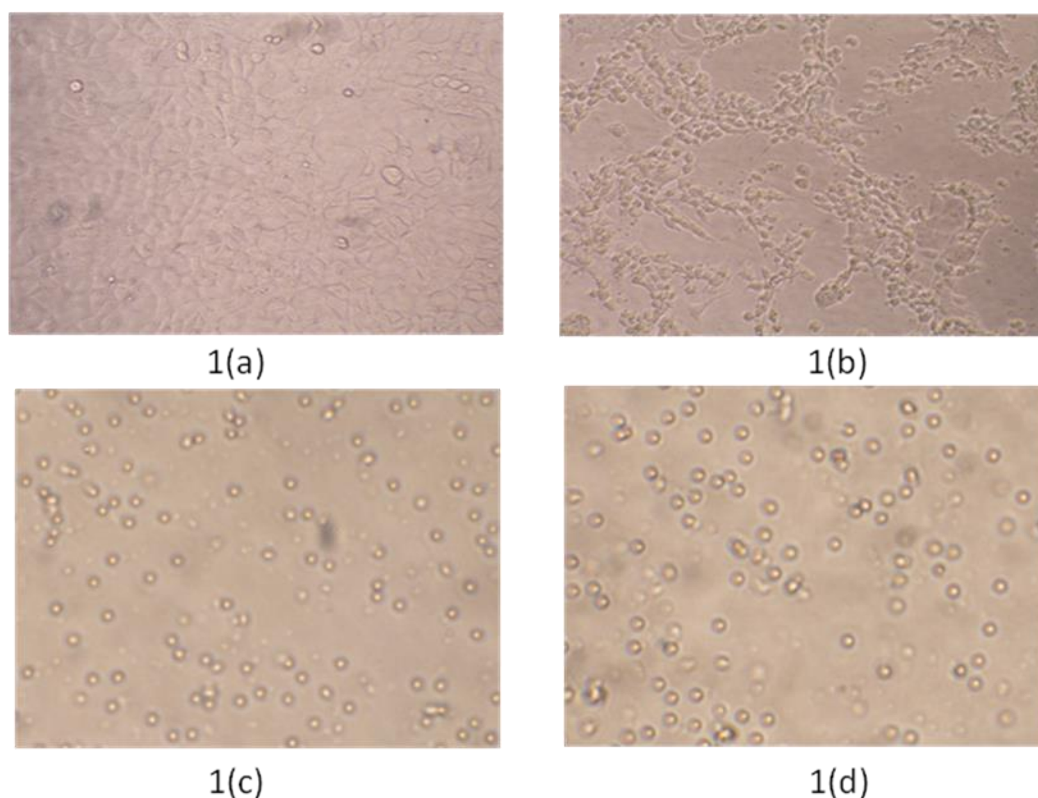

Figure 1: Photomicrographs showing the effect of Gallic acid. (a) Control HCT15 cells (untreated); (b) HCT15 cells treated with 96 µg/ml of gallic acid; (c) Control lymphocytes (untreated); (d) Lymphocytes treated with 96 µg/ml of gallic acid.

MTT Assay

In the present study, the MTT assay was used for the assessment of the cell viability. The gallic acid was tested for its cytotoxicity on the human colon cancer cell line HCT15 and peripheral blood lymphocytes. Gallic acid showed a dose dependent increase in cell death (IC_{50} of gallic acid was 96 µg/ml). However, the cytotoxic activity of gallic acid was not significant in peripheral blood lymphocytes with approximately 95% viable cells at the highest concentration (Fig. 2). This result is preliminary evident that the gallic acid has cytotoxic activity against cancer cells with no considerable effect on the lymphocytes. The subsequent experiments were performed at the IC_{50} value of gallic acid (96 µg/ml).

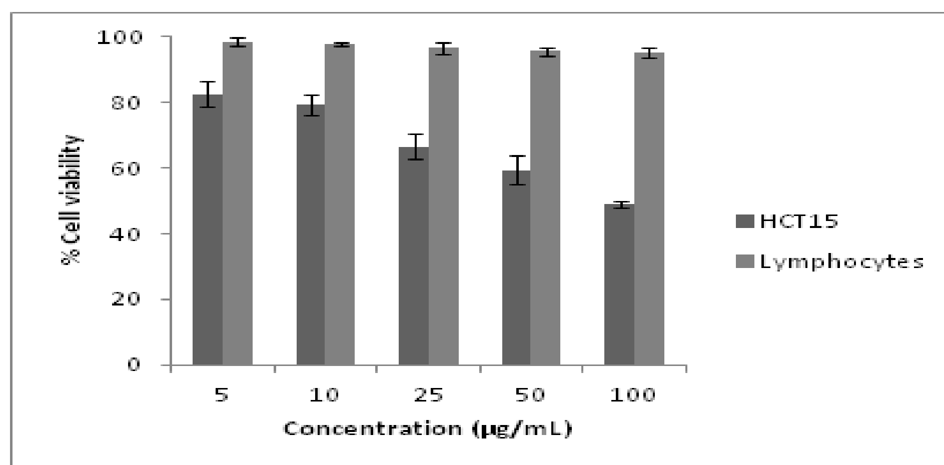

Figure 2: Cell viability assay of gallic acid on HCT15 and Lymphocytes. Data represents the mean +/- SD, n = 5.

COMET Assay

The comet assay was performed to analyse the DNA damage caused by gallic acid (96µg/ml). The exposure of HCT15 cells to gallic acid showed substantial DNA damage with the tail length 68.75, percentage DNA in tail of 17.75 and olive moment of 20.08 when compared to the control untreated cells. In contrast, the lymphocytes did not have significant DNA damage. Treated lymphocyte illustrated tail length of 3.83, percentage DNA in tail of 2.09 and olive moment of 0.82 which was relatively comparable to the untreated control lymphocytes (Fig 3; table 1). This finding indicates that gallic acid induced substantial DNA damage to the cancer cells while having minimum effect on the normal lymphocytes.

Table 1: Effect of gallic acid on the extent of DNA damage on HCT15 and lymphocytes

	Concentrations (µg/ml)			
	HCT15 Control	GA on HCT15	Lymphocytes Control	GA on lymphocytes
Tail length	34.33+/-4.16	68.75+/-3.09	2.90+/-2.11	3.83+/-2.87
% Tail DNA	11.33+/-2.20	17.75+/-1.83	1.69+/-0.96	2.09+/-1.25
Olive moment	7.39+/-1.64	20.08+/-2.28	0.60+/-0.46	0.82+/-0.53

The results are expressed as mean and standard deviation, n = 3. *P*<0.0001.

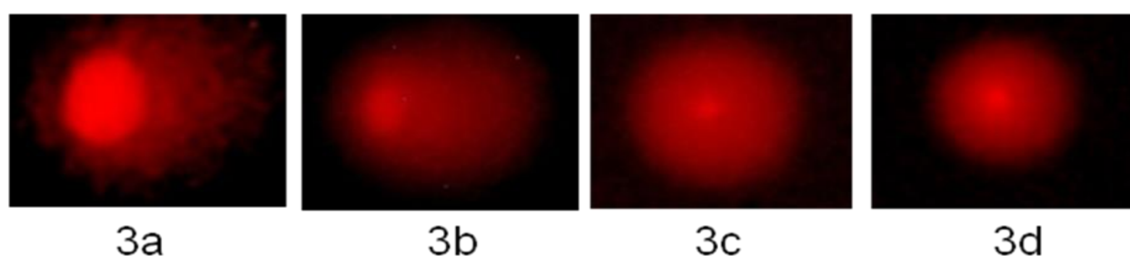

Figure 3: Comet assay showing DNA damage by gallic acid in HCT15 and lymphocytes, (a) untreated HCT15, (b) HCT15 treated with gallic acid (96µg/ml), (c) untreated lymphocyte and (d) lymphocyte treated with gallic acid (96µg/ml).

DISCUSSION

Present scenario in chemotherapy stipulates selective killing of cancer cells [14]. Hence, the effect of the anticancer agent should be limited to the cancer cells and not the normal cells of the body. Several studies on the effect of phytochemicals on numerous cell lines and animal models have illustrated the potential of phytochemicals in preventing cancer [15]. Gallic acid, being of the polyphenols having anticancer activity on vast number of cancer cell lines, was considered for the comparison of its effect on cancer and normal cells.

The MTT assay, a gold standard technique used for checking the cell viability, revealed that gallic acid has potential anticancer activity against HCT15 cells and minimal effect on the lymphocytes. Moreover, as membrane blebbing and DNA fragmentation is considered the hallmark of apoptosis, the HCT15 cells were believed to undergo apoptosis. This assumption was further supported by the comet assay which illustrated the extent of DNA fragmentation in the cells. Comet assay is a rapid and reliable technique and among all the parameters estimated tail length, percentage DNA in tail and olive moment are the most vital [16]. The extent of DNA damage was prominent in the gallic acid treated HCT15 cells in contrast to the lymphocytes which did not elucidate substantial DNA damage.

CONCLUSION

Thus, the outcome of the comparative study was that gallic acid is a potential anticancer agent as it targets the cancer cells without having any detrimental effect on the normal lymphocyte cells. This study can further be carried out at a molecular level to find out the possible mechanism involved in this contradictory effect on cancer cells and normal cells.

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